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INFLUENCE OF LONG-TERM EXPOSURE TO ADVERSE ENVIRONMENTS ON ORGA--ETC(U)
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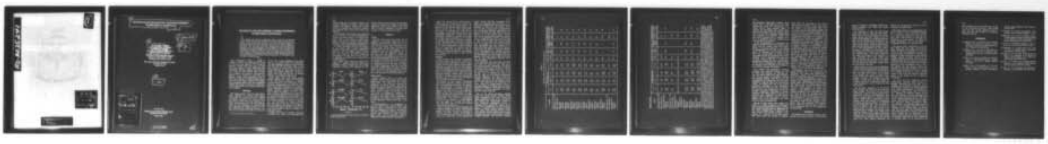

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INFLUENCE OF LONG-TERM EXPOSURE TO ADVERSE ENVIRONMENTS ON ORGAN WEIGHTS AND HISTOLOGY.

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INFLUENCE OF LONG-TERM EXPOSURE TO ADVERSE ENVIRONMENTS ON ORGAN WEIGHTS AND HISTOLOGY

A comparison was made of the morphologic effects of cold, heat, and simulated altitude on adult male rats given exposures of 24 weeks' duration. By the use of covariance analysis it was possible to determine the extent to which organ weights were dependent on body weight and to adjust the values in order to remove influences of body weight. For liver, heart, and kidney, adjusted weights indicated temperature-dependency, while pressure-dependency was established for liver and kidney only. Histologically, temperature-dependency was indicated for liver, kidney, thyroid, adrenal, and pituitary. Fur weight was reduced in heat but not altered in cold. Fasting in cold induced changes in adrenal and thymus weight and unusually high body weight loss; in heat, fasting caused a significant thymus weight loss without adrenal weight increase. The thymus-adrenal ratio was elevated during a 24-hour fast in all environments except cold, where it was decreased.

The relationships between a number of metabolic indices and environmental factors in rats have been reported (1-3). Among the variables studied there were some for which there was virtually a linear relationship with temperature when the exposures were continued for as long as three months. Many of these same variables also proved to be pressure-dependent. As an extension of this work, morphologic studies were subsequently made on these same animals, and these findings constitute the present report. Primarily, the purpose was to determine whether there were morphologic changes which relate to pressure or temperature in the same manner as was noted among metabolic variables.

METHODS

Male Sprague-Dawley rats were caged individually, subjected to a standard daily lighting and animal care schedule, and fed ad libitum commercially prepared food mixtures (Purina Dog Chow or Red Chain Dog Checkers). Initially, body weights were in the 350 to 400 gm. range. In each experiment an original group was subdivided by random selection into four test (environmental) subgroups – namely, *cold* (3° or 5° C.), *neutral* (24° to 26° C.), *hot* (34° to 35° C.), and *altitude* (simulated, with temperature at the neutral level). In one experiment, two other subgroups (*altitude-cold* and *altitude-hot*) were used, but observations made on these latter groups are less extensive and are considered secondary to those made on the first-named four groups. Barometric pressure at ground level was 750 mm. Hg; to simulate altitude, it was reduced to 380 mm. Hg.

Organ weights (liver, heart, kidney, spleen, thymus, adrenal, and testes) were determined in the 24th week of exposure on randomly selected members of each environmental group. Histologic preparations were made from all of these organs and, additionally, from pituitary, thyroid, parathyroid, parotid and submaxillary glands, pancreas, tongue, and brain. Teeth were examined histologically and also chemically, but these data are reported elsewhere (4). Fur weights and Harderian gland weights were determined on animals other than those used for histologic studies. The effects of a 24-hour fast were determined on randomly selected rats from each of the four main groups (in their accustomed environments), and changes in adrenal and thymus and body weights were used as the basis on which to compare environmental groups.

Tissues were fixed in formalin, embedded in paraffin, and stained with hematoxylin and

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eosin except for the pituitary, kidneys, and adrenals. Pituitary and kidney sections were stained with Mallory's aniline blue. Frozen sections of adrenal were examined for birefringent material; other sections were stained with Sudan IV.

Most histologic characteristics were estimated on a scale of 0 to +4. Uniform dimensional estimates were made relative to the same microscopic field in any series of observations. Efforts were made to estimate relative tissue densities, cell and nuclear sizes, and proportional volumes occupied by important subdivisions.

To obtain fur weight, all rats were clipped by one person using a highly standardized procedure. Each fur sample was dried overnight at 37° C. and then weighed. The fur was quite clean in all groups except the heat-exposed; in this case it was "rusty" in appearance and parts were encrusted with epithelial debris.

To evaluate organ weight data statistically, analysis of variance (observed values) and covariance (values adjusted for body weight differences) technics were employed (5). Differences between mean values for environmental groups were tested with suitable t-tests where significant F-ratios were obtained. The group held at neutral temperature at ground level pressure was taken as the control group.

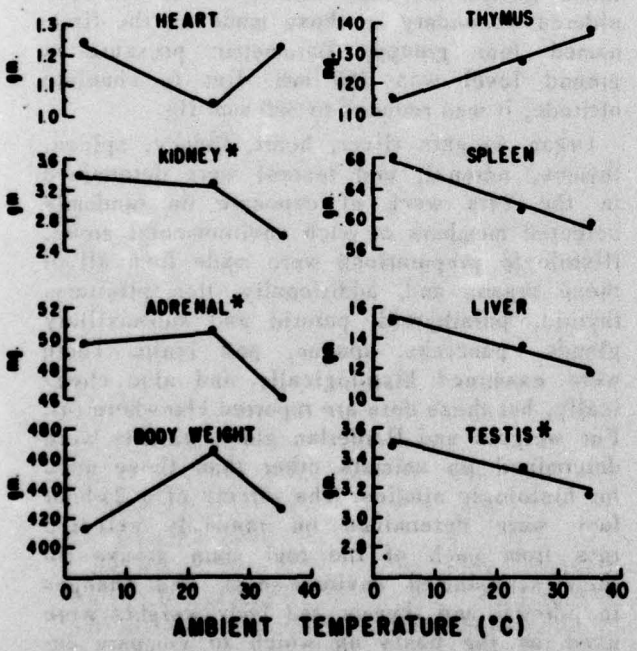

FIGURE 1

Temperature dependency of organ weights. The asterisk denotes weight per pair.

RESULTS

General

Although initial mean body weights for the subgroups in a particular experiment were not different, terminal values (table I) for the altitude and hot groups were consistently lower ($P \leq .05$) than control values. Terminal body weight values for the cold groups also ran consistently low, but the difference in experiment 4 was not significant. Despite the fact that they were lighter in weight than the controls, fat accumulations were found in all the heat-acclimated rats, whereas the cold-acclimated and altitude-acclimated rats, which were lighter also, had little or no mesenteric or subcutaneous fat. In the latter two groups the external appearance had been in no way indicative of poor health. The same statement cannot be made for the heat-exposed rats with their "rusty" fur and hyperemic paws, ears, and scrota and dirty, encrusted, mucoid tails.

Organ weights

Graphic analysis (fig. 1) of these data (adjusted values only) revealed almost linear relationships with temperature for thymus, spleen, liver, and testes and nonlinear relationships for heart, kidney, and adrenal. Statistical analysis (table I) established that chance variations could not be ruled out for thymus, spleen, testes, and adrenals, but for liver, heart, and kidney, the variations with temperature were statistically significant. In the case of the kidney, however, temperature-dependency was limited to the upper part of of the temperature range studied (fig. 1 and table I).

Pressure-dependency was established for liver and kidney – each varying directly with pressure (table I, experiment 1). Fur weight (table I, experiment 2) was not increased in cold, but a significant reduction in fur weight resulted from exposure to heat. Harderian gland weight was not influenced either by temperature or pressure (table I, experiment 3).

Mean body weight losses resulting from a 24-hour fast were similar in the neutral and hot environments and at altitude (table I, experiment 4), and adrenal gland weights in the fasted animals did not differ significantly from those of nonfasted rats. (The rats in experiments 1 and 4 were drawn from the same experimental populations.) Fasting in the cold environment resulted in a greater body weight loss which was accompanied by an increase in adrenal weight and a decrease in thymus weight. Although adrenal weight did not change in the other groups, thymus weight increased in the neutral environment, increased slightly (but not significantly) at altitude, and decreased in both cold and heat. In each environment the thymus-adrenal ratio was changed by a 24-hour fast, but only in cold was the value lowered.

Histology

Brain sections were examined for necrotic foci and tongue sections for evidence of vitamin deficiency with negative results. Tongue sections for the altitude animals showed marked hyperemia, as expected, but in no other respect were they different. In cold, but not elsewhere, parotid and submaxillary gland sections showed signs of low-grade, chronic inflammation and occasional calculi, occluded ducts, and cysts. Except for a tendency toward pale-staining and cuboidal cells in sections from cold-exposed rats, all parathyroids seemed normal.

Indications of mild, chronic inflammation were noted in pancreas sections from all rats, but no environmental specificity was discernible. The hyperemia in sections of pancreas from altitude-exposed rats permitted their recognition, but that was the only difference. Spleen sections from cold-exposed rats contained more than the normal amounts of hemosiderin along with indications of very active hematopoiesis. The degree of hematopoietic activity in the spleens of altitude-exposed rats was judged to be greater than that in cold, but there was no difference between neutral and hot environments.

Liver sections from altitude-exposed rats exhibited variability in nuclear characteristics greater than that in the controls. There was a slightly decreased cellular organization in liver sections from both altitude- and cold-exposed rats. Additionally, altitude-exposed rats showed dilated sinusoids. Cytoplasmic basophilia was depressed and the number of binucleate parenchymal cells was elevated (two- to threefold) in cold, heat, and altitude. About twice as many ectopic sites of hematopoiesis and intravascular nucleated erythrocytes were noted in the cold and four times as many at altitude as in the control. (Liver sections from rats in altitude-cold contained no ectopic sites of hematopoiesis; however, the increase in the number of nuclei per cell was equal to that in cold alone.) The relative lobular area and average cell size in liver sections from cold-exposed rats were estimated to be about 20 percent greater than in the controls, while there was about a 15 percent reduction in the heat-exposed rats. Amyloid and albuminous degeneration was seen occasionally in all environments, but such changes were more frequent in the heat. Only in this latter group was any fatty degeneration observed, but it was not extensive.

The impression was gained that myocardial fiber width was greater in cold and less in heat than at neutral temperature. The number of circular fibers in cross sections per high-dry microscopic field (average of 10 rats, 3 counts per section) were 23.6 in the cold, 27.8 in the heat, 23.5 at altitude, and 24.8 in the controls. Only the heat differed significantly from the controls (F 4.73 with 36 degrees of freedom). Calcinosis was observed in heart sections from rats in all environmental groups, but it was localized to the periphery in all cases except the altitude-exposed, where it was more diffuse. The altitude rats also showed widespread albuminous degeneration and a high incidence of fibrosis. Hyperemia, of course, was very evident at altitude.

Most of the kidney specimens, including those from control animals, exhibited diffuse hyaline degeneration with some sclerosis and highly angiomatous stroma in the corticomedullary junction area. Occasionally, evidence of mild, nonspecific inflammatory activity accompanied these degenerative changes, but in none of the environments was there sufficient damage to indicate serious functional impairment. Along with hyperemia, kidney sections of altitude rats showed isolated areas of ectopic hematopoiesis, and, in scattered areas, swollen, irregular glomeruli with increased cellularity

TABLE I

Statistical analysis of body and organ weight data

Variable	Environmental group				Error	Terms	Regression coefficient (within groups)	Significant F-ratios	
	Cold	Neutral	Hot	Altitude	$\sqrt{MSw}$	$\sqrt{MSw}$ (adjusted)		Analysis of variance	Analysis of covariance
Experiment 1 (n = 10)									
Body weight (gm.)	413*	464	426*	388*	30.2	–	–	11.	–
Liver (gm.)									
Observed	15.2*	14.1	11.7*	12.1*	1.08	.953	22.8	24.	22.
Adjusted	15.2*	13.4	11.7*	11.5*					
Heart (gm.)									
Observed	1.28	1.22	1.24	1.20	.160	.084	4.52	NS	17.
Adjusted	1.24*	1.03	1.22*	1.05					
Kidneys (gm.)									
Observed	3.41	3.41	2.61*	2.63*	.319	.319	5.78	21.	14.
Adjusted	3.38	3.24	2.59*	2.50*					
Spleen (gm.)									
Observed	0.68	0.64	0.60	0.69	.110	.109	0.53	NS	NS
Adjusted	0.68	0.61	0.59	0.66					
Testes (gm.)									
Observed	3.54	3.59	3.17	3.14*	.435	.437	4.55	2.98	NS
Adjusted	3.49	3.34	3.14	2.93					
Adrenals (mg.)									
Observed	49.7	50.5	46.1	52.5	9.13	9.35	21.9	NS	NS
Adjusted	49.7	50.4	46.1	52.4					
Thymus (mg.)									
Observed	122	159	141	161	47.9	42.1	495	NS	NS
Adjusted	115	127	137	135					
Thymus/adrenal									
Observed	2.44*	3.15	3.06	3.06	.189	–	–	3.62	NS
Adjusted	2.32	2.52	2.98*	2.57					
Experiment 2 (n = 15)									
Body weight (gm.)	351*	404	352*	359*	51.6	–	–	5.86	–
Fur weight (gm.)									
Observed	5.20	5.61	4.30*	4.95	.704	.704	5.10	13.	10.
Adjusted	5.16	5.53	4.26*	4.90					

TABLE I (Contd.)

Variable	Environmental group				Error	Terms	Regression coefficient (within groups)	Significant F-ratios	
	Cold	Neutral	Hot	Altitude	$\sqrt{MSw}$	$\sqrt{MSw}$ (adjusted)		Analysis of variance	Analysis of covariance
Experiment 3 (n = 20)									
Body weight (gm.)	361*	406	314*	359*	.0374	–	–	20.	–
Harderian gland (gm.)									
Observed	.314	.382	.340	.324	.106	.098	.84	NS	NS
Adjusted	.302	.329	.287	.312					
Experiment 4 (n = 10)									
Body weight (gm.)	381	416	367*	356*	45.2	–	–	3.33	–
Fasting weight loss (gm./24 hr.)									
Observed (percent loss in *parentheses*)	45.2*(11.9)	27.1(6.5)	23.9(6.5)	23.3(6.5)	3.60	–	–	4.76	
Adrenals (mg.)									
Observed	56.5*†	49.4	44.9	51.0	5.57	5.60	26.3	34.	33.
Adjusted	55.7*†	39.0	37.8	42.8					
Thymus (mg.)									
Observed	104*†	212†	128*†	160*	51.3	46.6	643	8.34	7.22
Adjusted	102*†	194†	122*†	147					
Thymus/adrenal									
Observed	1.83*†	4.29†	2.86*†	3.13*	.126	–	–	4.71	NS
Adjusted	1.84*†	4.96†	3.22*†	3.44*†					

The value given for each group is the mean. For a given experiment the number of animals in each group is equal. Weights given for kidneys, testes, and adrenals are for the pair. Since the variances were homogeneous throughout (Bartlett's test, 5), the square root of the *Mean Square Within Cells* ($\sqrt{MSw}$) provides an estimate of the average standard deviation. Analysis of covariance of organ and body weights was performed in each experiment along with analysis of variance of observed values. The significant F-ratios and the error terms ($\sqrt{MSw}$) of both analyses are presented. The mean values were adjusted to account for the influence of body weight ($Y - b_w X = Y'$), where Y is the deviation of the observed organ weight, b_w is the regression coefficient within groups, X is the deviation of the body weight, and Y' is the adjusted organ weight. Suitable t-tests (with $\sqrt{MSw}$ used as the error term) were made where the F-ratio was significant, and an asterisk signifies that the mean is significantly different from the neutral control group ($P \leq .05$). In experiment 4 a dagger indicates that the value in the fasted group differs significantly ($P \leq .05$) from the nonfasted (compare values in experiments 1 and 4).

and albuminous degeneration of tubules. Renal tubule cells in cold-exposed rats were characteristically large and pale-staining, containing uniform, often vesicular, nuclei; in contrast, the heat-exposed rats showed small, dark-staining tubule cells with irregular, dense nuclei. In both groups albuminous degeneration and dilatation in convoluted tubules were found more frequently than in controls; but it is to be emphasized that such conditions were observed also in the controls.

In the adenohypophysis of cold-exposed rats enlarged basophils were found frequently. Compared with the controls, there was approximately a 50 percent reduction in the number of chromophobes in this same group, and the number of basophils was increased several fold. Cell size generally was reduced in the heat-exposed group. (In altitude-heat this effect appeared to be intensified – the cells were not only small but they had a "washed-out" appearance.) Nuclear size was highly variable in heat (and in altitude-heat), and there was an increase in the percentage of chromophobes and a relative decrease in acidophils.

The variation with temperature in thyroid histology was especially striking. For cold at ground level pressure, the histologic picture was typical (small acini, high epithelium, and reduced amounts of irregularly staining and scalloped colloid), while that in heat was predominantly the storage type with low acinar epithelium, relatively large acini and plentiful colloid. The glands from rats at the neutral temperature represented a state intermediate between those in heat and cold. (At altitude the picture in cold seemed more extreme and included lymphocytic infiltration; at neutral temperature the mixed patterns of activity and inactivity were reminiscent of early nodular change; while in heat the degree of activity appeared not to be depressed to the same extent as in heat at ground level.)

Adrenal sections (either those stained with Sudan IV or those examined for birefringent material) showed considerable variation. Average cell counts indicated that the glomerulosa in the controls occupied about 15 percent of the total cortical cross section, whereas it was reduced to 6 percent in the other groups (the lowest value was obtained in altitude-cold). Where the fasciculata in the controls amounted to 64 percent of the total area, it was reduced to levels varying from 46 to 54 percent in the experimental groups. The reticularis, which occupied approximately 21 percent of the area in controls glands, was increased to some degree in each of the adverse environments. With respect to cytoplasmic and nuclear mass, cold and heat appeared to act differently – cold caused increases in both cytoplasmic and nuclear mass, while heat acted to increase nuclear but not cytoplasmic mass. Birefringent material in control glands tended to appear as finely to moderately granular masses distributed throughout the fasciculata; in cold the tendency was toward massive accumulations of coarse material in the glomerulosa and reticularis with little or none in the fasciculata; in heat, coarse aggregates appeared throughout the fasciculata but not elsewhere. (At altitude, either at neutral temperature or in cold, the distribution and character of birefringent material tended to be normal, but in altitude-cold there was an increase in the coarseness of the aggregates.)

Histologically, the testes of the four main groups showed little or no peculiarities (no specimens were taken from altitude-cold or altitude-hot groups). The germinal epithelium had the typical arrangements in all environments, and although individuals showed some cytolysis and fragmentation with lumina containing debris, the experimental groups showed this tendency only to a slightly greater degree than the control. Waves of activity were evident in all specimens, and mature spermatozoa in high numbers were present in all. Evidence suggestive of an environmental effect was found in only two specimens from the heat group. In these, Sertoli cells were especially prominent in a few seminiferous tubules, but there were spermatozoa attached to them, and between them and the periphery, mitotic figures were quite numerous. This suggests that a period of inactivity had occurred, as in the cryptorchid state, but that regeneration was proceeding.

DISCUSSION

The differences in final body weight occurred because the controls continued to gain slowly,

while the weight of experimental animals plateaued. Individual rats which lost weight eventually died.

The covariance of organ and body weight varied from organ to organ and with environments; thus, it can be concluded that environmental factors had direct effects on certain organs. These findings are in general agreement with those of Herrington and Nelbach (6), who found, by the use of correlation technics, that organ to body weight or organ to organ relation ships became distorted during exposure to either hot or cold environments and that negative correlation coefficients for organ pairs were more frequent in the adverse conditions than at thermoneutrality. This latter observation was taken as evidence of an approach to a final equilibrium state. Their studies were made on growing rats, whereas in the present case the rats were nearly grown at the outset; for this reason, the particular results cannot be expected to be the same.

Organ weights may vary because of differences in tissue structure, extracellular or intracellular water or fat, or differences in blood content. Since the higher blood content of the organs of the altitude rats would be a factor which would increase their weight, provided no other changes occurred, the finding that organ weights in these rats were either similar to or lighter than those in controls indicates that tissue constituents or water content must have been reduced.

The histologic appearance of the tissues from individual rats within any one group was highly variable. These data were not treated statistically, so the conclusions represent semiquantitative estimates and impressions. Possibly, a greater degree of uniformity would indicate a high degree of adaptation. In those animals which were exposed to combinations of thermal and pressure factors the variability seemed especially high. The variations in liver and kidney in cold and heat were contrasting ones: in cold, cells tended to be large with pale cytoplasm and uniform, large, vesicular nuclei; in heat, cells tended to be small and to have dense cytoplasm and deeply staining nuclei of variable size. The contrast is similar to that between young cells and old cells. For other reasons Herrington and Nelbach (6) considered the effect of heat to be similar to an aging effect.

The present results are in disagreement with those of Gilson (7), who reported that exposure for 16 weeks to cold of this degree resulted in no organ weight differences except for adrenals, which were heavier, no histologic abnormalities in visceral organs, no evidence of thyroid hyperplasia, and no abnormality in sudanophilic material in the adrenal cortex.

Few reports permit comparisons of cold and heat. While cold may induce adrenal hypertrophy, according to Tepperman et al. (8) this is not the case during exposure to heat. The present results are confirmatory. Bernstein (9) reported a normal distribution of adrenocortical lipoidal material in adrenal cortices of rats exposed to heat; however, there were indications of thyroid inactivity. The increase in the number and in the size of the basophils in the anterior pituitary of the cold-exposed rats is not a new finding, but the contrast between the histology in cold and heat seems not to have been reported previously. The lack of change in fur weight in the cold is in line with the report by Hart (10) that insulative adaptation occurs in the natural but not in laboratory environments.

As Selye (11) has emphasized, diverse environmental conditions induce similar morphologic alterations but these may be accompanied by or modified by specific effects. In this study, members of an original population were studied concomitantly in dissimilar environments. Chance factors thus were reduced to a minimum and important factors such as diet, age, duration of exposure, lighting schedule, etc., were precisely controlled. While some of the changes (especially those in the heart, kidney, and adrenal) resemble "general adaptation syndrome" effects described by Selye, the variations with temperature suggest specific effects. The generally employed criteria of adaptation were satisfied since the animals were not losing weight and, histologically, they had normal reproductive capacity. Some of the tissue changes may be interpreted as age effects, and possibly the adverse factors acted to accelerate these effects. Gross examination of visceral organs of all nonsurvivors was

done routinely, but the best that can be said is that nonsurvivors had had a body weight loss and poor external appearance prior to death and that the incidence of pulmonary infections was high.

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